

A Study of the Sampling and Assay
Methodology necessary to determine the
Mutagenic Potential of Diesel Exhaust
Particulate by Ames Salmonella Bioassay

ORF 81-1

FINAL REPORT

by

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Work on this project was conducted under the auspices of
Canada Centre for Mineral and Energy Technology,
Energy, Mines and Resources, Canada.

March 31, 1981

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63pp

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DSS File Number: 23SQ.23440-9-9178
DSS Contract Serial Number: 1SQ80-00045
ORF Contract Number: 11115

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SUMMARY

The particulate emissions from a Deutz F6L 714 diesel engine, commonly used in underground mines, under a fixed load speed condition (2200 RPM, 7/8 load) with and without a catalytic converter fitted, were collected by three different methods - a 47 mm filter sampling system, a 125 mm filter sampling system and a dilution tube sampling system. The 125 mm sampling system and a modified dilution tube method using a Hi-Vol sampler proved to be the most practical methods for collecting the fairly large amount of particulates required for Ames testing. The soluble organic fractions (SOF) of the collected particulates extracted with dichloromethane (DCM) followed by solvent exchange into dimethyl sulfoxide (DMSO) were subjected to Ames assays using five strains of Salmonella. All samples tested in the Ames assay showed a negative response to tester strain TA 1535. All samples tested showed a positive mutagenic response to tester strains TA 1537, TA 1538 and TA 98 both with and without metabolic activation. Tester strain TA 100 showed a variable positive/negative response which is not unexpected when using unfractionated samples with this strain. The results obtained with a PTX catalyst equipped engine were slightly more mutagenic than those obtained without catalyst.

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I INTRODUCTION

Concern over the potential mutagenic, and by implication, carcinogenic activity of diesel generated particulate matter in underground mines, has led to a requirement to identify the magnitude of this potential health hazard, and possible control techniques to reduce mutagenic activity.

To chemically identify the variety of hydrocarbons with potential cancer causing activity in diesel exhaust is a formidable task. However, a method exists for the rapid screening of such material for mutagenic activity in the form of Ames mutagenicity assays. This technique for assessing mutagenicity of diesel particulate matter is recognized by the EPA in the United States to offer the best potential for providing an analytical tool to identify the nature and extent of this potential hazard, but their work on emissions has not addressed the special problems surrounding the testing of diesel exhaust emitted from typical derated underground diesel engines. Furthermore, it is estimated that over 90% of Canadian mines diesels and 60% of U.S. mines diesels employ catalytic converters as exhaust treatment devices. These catalysts are recognised to effectively oxidize carbon monoxide and light hydrocarbons such as methane to CO_2 but they also create the opportunity for oxidizing SO_2 to sulphuric acid. The sulphuric acid production is a negative factor on the desirability of using catalysts on underground diesel powered machinery. Continued use of catalysts in this regard would, however, be highly desirable if it was found that the catalyst was capable of oxidizing hydrocarbon material to the extent that it reduced mutagenic activity as measured by the Ames test.

Before catalyst or other control devices such as exhaust gas filters, water/fuel emulsion techniques, can be evaluated using the Ames test, it is necessary to develop the basic methodology for underground, catalyst treated, heavy duty diesel exhaust so that meaningful results are obtained. It is recognized from work by the EPA and from experience at Ontario Research that meaningful results are only obtained by carefully

addressing both the exhaust sampling techniques and mutagenic assay techniques in order to select the most appropriate methodology.

It was with this background that this work was undertaken with the following objectives:

II OBJECTIVES

1. To examine different exhaust sampling techniques, with a catalyst and non-catalyst equipped engine, on Ames test assay results using (a) a 47 mm filter assembly similar to that employed at the Explosive Atmospheres Laboratory of CANMET; (b) a larger 125 mm filter assembly similar to that employed in EPA studies for source sampling in which the linear velocity of the exhaust gas through the filter would be reduced compared to the 47 mm assembly; and (c) sampling of a diluted exhaust stream in order to simulate as closely as possible the situation underground and which would allow study of the effect of dilution ratio.
2. To examine different mutagenic assay techniques for a given sample to determine the most rapid and inexpensive techniques to produce the most meaningful results.
3. To select the most promising methodology suitable for the mines diesel and to compare the results with the results obtained by EPA on a well documented and widely tested engine, the G.M. Oldsmobile diesel.

1. DIESEL ENGINE TEST REQUIREMENTS

The engine used for this test programme was a Deutz F6L 714 diesel engine, rated at 101 kw at 2300 RPM. This engine is air-cooled and incorporates a pre-combustion chamber for indirect injection of diesel fuel into each cylinder. Lower exhaust emissions are characteristic of indirect injection compared to direct injection engines of similar horsepower ratings. Deutz engines, therefore, are in common use in underground mines at the present time.

The engine selected for this particular programme, Serial No. 4 841 927, was used in previous CANMET and other programmes. In the course of some of these programmes the engine was subjected to water/diesel and methanol/diesel emulsions as well as exhaust gas recirculation. It was felt that the condition of the engine should be evaluated prior to any testing to ensure that results would conform to a "typical" mines engine. The injection pump and injectors were removed and delivered to a local repair facility. The injectors required replacement and the fuel pump required new plungers and barrels. All units were returned to ORF meeting manufacturer's specifications and re-installed in the engine.

A compression check was performed on the engine with results showing cylinder pressures near the lower end of the 25 to 31 bar specification by Deutz. The cylinder heads were removed and inspected revealing an improper seating pattern on the cylinder liner. Elongated studs were suspected and removed and replaced with new parts. Further checks showed improved cylinder pressures.

For logistical reasons emissions testing on the refurbished engine was conducted under a separate CANMET contract during the initial phase of this programme. This other CANMET contract involved the evaluation of an exhaust treatment device. While testing this device, the engine malfunctioned and required complete removal and inspection by Deutz.

It was determined that this malfunction was caused by a piston "meltdown" on cylinder No. 4. The engine was repaired and re-installed for continuation of testing under this programme. Further testing under the other CANMET contract was postponed until this programme was completed.

Gulf Diesel 40 was used as a fuel for all tests. This grade of fuel is typical of that used in underground mining operations. Fuel specifications are as follows:

<u>Component</u>	<u>Before Engine Malfunction</u>	<u>After Engine Repair</u>
Carbon %	84.8	86.7
Hydrogen %	14.4	13.3
Sulphur %	0.16	0.16
Heating Value BTU/lb	-	19720
Specific Gravity	0.8251	0.8259

All engine testing was conducted at 2200 RPM and 86 kw (7/8 load). CANMET has suggested that this condition is representative of the time weighted average of the load-haul-dump cycle of a mining scoop tram. A Go-Power DT1000 dynamometer was used to control load on the engine during emissions tests.

A number of particulate tests were performed with emissions controlled using Engelhard PTX 60F monolithic catalysts. Two catalysts were used, one on each engine bank, with catalysts mounted as close as possible to the exhaust manifold for optimum performance. These catalysts are 15.2 cm in diameter and 7.62 cm in depth which produces an actual space velocity of $4.74 \times 10^6 \text{ hr}^{-1}$ in each catalyst at the engine condition evaluated. The exhaust temperature at the catalyst inlet generally averaged 477°C for the 2200 RPM/86 kw engine condition. Typical performance of the catalyst, determined in earlier programmes at ORF, is shown in Table I.

2. SAMPLING SYSTEMS AND METHODOLOGIES

2.1 Background

The sampling and characterization of diesel exhaust particulate emissions to achieve representative sampling and reproducible results is complex and is continually being upgraded. Optimum methods for determining the fractions of carbon black, condensible organics and sulphuric acid which can comprise diesel particulate have not been fully established. It is well known, however, that sampling methodologies and conditions can greatly affect the collection of any of the above fractions. For example, filter temperatures in excess of $\sim 60^{\circ}\text{C}$ will cause loss of the soluble organic fraction (SOF) collected on the filter. Also, sampling train probes can retain a considerable portion of the exhaust gas sample (particularly the SOF) and this fraction must be included in any subsequent analysis and data presentation. When catalysts are used to control diesel engine emissions considerable amounts of sulphuric acid can be generated and significantly increase the total mass of material collected on the filter.

As the principal work in this programme involves the sampling and analysis of particulate matter for Ames mutagenic activity, it is clear that any of the factors described above could affect the Ames test in one way or another. For example, during the collection of particulate matter on a filter, the particulate is exposed to exhaust gases passing through the filter during the period of sample collection. NO_x , in the exhaust, could conceivably allow nitration reactions on the particulate organics to proceed, which will affect Ames activity.

This programme, therefore, included the evaluation of three diesel exhaust sampling systems in order to examine the effect of sampling methodology on Ames activity. Two of these systems extracted a portion of the raw diesel exhaust from the exhaust pipe with particulates collected on an appropriate filter. In one case a 47 mm filter was used, while in the other, a 125 mm filter was used. The third sampling method consisted of extracting a portion of the raw exhaust, diluting

the exhaust with air, then collecting the diluted exhaust sample on a filter. Dilution sampling methods are frequently used in sampling diesel particulate since they can allow for chemical mechanisms and agglomeration of particulate material to occur before collection on the filter. This method tends to simulate real-world conditions when diesel exhaust leaves the tailpipe. A number of studies have been conducted regarding extractive dilution techniques^{1,2,3,4}.

The following sub-sections describe the sampling systems used. Some modifications were required in each system.

2.2 47 mm Filter Sampling System

This system is used extensively by CANMET for certification of emissions of underground mines diesel engines. The initial system design for this project consisted of a 2.5 m by 6.35 mm diameter Teflon tube connected to a 47 mm stainless steel filter holder. Samox heat tape was wrapped along the length of the Teflon line to minimize losses due to condensation. A diaphragm type pump was used to extract the sample and a calibrated dry gas meter measured the volume of gas sampled. Greenburg-Smith impingers were used to condense moisture in the sample gas between the filter and vacuum pump. Figure 1 illustrates the sampling system.

In order to facilitate rapid removal of filters, six holders were fitted with quick-disconnect fittings. These six filter holders were placed in an oven to allow each to condition uniformly. An oven temperature of 41°C was determined to be the lowest possible sampling temperature for maximum organic collection and still be above the dewpoint of the exhaust gas stream. A thermocouple, installed directly in the 47 mm filter holder, allowed for the measurement of the gas temperature.

Several tests were performed in this configuration and results showed the filter temperature increased from 38°C to 67°C in 4 minutes. During these tests the Teflon line was maintained at 54°C. Following this, the sample line heat was turned off and tests repeated. Similar temperature increases were noted over a period of 15 minutes. It was expected that sampling time for each filter would be approximately

3 minutes and that the filter temperature rise would not be greater than 43°C. To verify this, a series of filters were tested without sample heat. Each filter was allowed to sample for 3 minutes and 35 seconds were required for filter changeover. Results showed that filter temperatures increased from 40°C to 53°C for the first filter, 51°C to 58°C for the second, and 50°C to 59°C for the third filter. The problem of inconsistent filter temperatures remained.

Sampling with the Samox heat tape removed showed filter temperature increases from 40°C to 52°C in a period of 4 minutes. A thermocouple was used to determine the point on the Teflon sample line where the line skin temperature was 42°C. This point was then located at the oven entrance which allowed for the balance of the sampling line (~0.5 m) to be placed inside the oven. It was felt that this would allow sufficient distance at oven temperature to provide consistent filter temperatures. This configuration was evaluated for a 10 minute test at a 0.023 m³/min sampling rate with the filter temperature varying between 41°C and 42°C. It was felt, however, that the 30 second filter change-over period would allow for condensation of moisture in the sample line.

Next, a water cooled probe was designed to maintain uniform sample gas temperature and provide an adequate heat sink to prevent condensation during filter changeovers. The 2.5 m by 6.35 mm diameter Teflon sample tube was placed inside a 2.0 m by 10 mm diameter polypropylene tube. With appropriate fittings the water was introduced in a counter-current direction to ensure stable sample gas temperatures. An insulated container was used as a water reservoir and a Brinks pump was used to recirculate water through the system.

Tests were conducted with the water cooled probe with the engine injection timing set to what was thought to be 27° BTDC. Further investigation showed the timing was closer to 30° BTDC. Results of these tests showed that the average total particulate loading, with the modified 47 mm system, was 25.7 mg/m³.

The injection pump was re-set to 24° BTDC and three test series performed during which 52, 35 and 20 Pallflex filters respectively were used for particulate collection.

During Series 1, 52 filters were collected with the filter temperature at 43°C. However, during this test series, it became apparent that some of the soluble organic fraction (SOF) of the particulate was being lost in the sample line. This was obviously unsatisfactory, as the SOF is a major contributor to Ames mutagenic activity. Accordingly, Series 2 was run with the sample gas temperature at 141°C in order to try to minimise condensation of organics in the sample line. This was unsuccessful, as was Series 3 carried out at a sample temperature of 66°C.

It was clear that in order to collect the total SOF for Ames testing, a technique must be employed which permits the SOF to be extracted from the sample line and added to that collected on the filter.

2.3 125 mm Filter Sampling System

Following the 47 mm filter tests, a 125 mm filter sampling system was evaluated. The sampling system is shown in Figure 2. Stable filter temperature results achieved with a water-cooled probe on the 47 mm sampling system prompted its use on the 125 mm system. The Teflon line, however, was replaced with a 1.5 m long stainless steel probe to allow for rinsing of the probe after each test. Identical water re-circulation equipment was used on the 125 mm system.

Since the Ames mutagenic tests would be conducted on the 125 mm samples, a blank test run was performed. All glassware and surfaces in contact with the sample gas stream were thoroughly cleaned and rinsed with dichloromethane. The sampling train was then operated in the laboratory at a sampling flow rate of 0.023 m³/min for 3 hours. The train was then rinsed with dichloromethane and the Pallflex filter removed. Both the rinsings and filter were analyzed by the Ames Assay to determine blank values.

Tests were then conducted on the Deutz engine exhaust at a sampling flowrate of $0.014 \text{ m}^3/\text{min}$. This allowed for approximately the same sampling flowrate per square centimeter of filter area as found for the 47 mm filter tests ($0.242 \text{ m}^3/\text{min}/\text{cm}^2$ for the 125 mm filters and $0.207 \text{ m}^3/\text{min}/\text{cm}^2$ for the 47 mm filters). However, the time required to obtain 300 mg of sample would be approximately 18 hours at the sampling flowrate of $0.014 \text{ m}^3/\text{min}$. The sampling flowrate was therefore increased to $0.023 \text{ m}^3/\text{min}$ which reduced the total sampling time to 9.5 hours for the collection of 300 mg of particulate.

Three tests were conducted for the five strain mutagenic assay, designated Tests Nos. 1, 2, 3 Raw, respectively. Test Nos. 1 and 2 Raw were conducted on uncontrolled emissions while Test No. 3 Raw was performed with the Engelhard catalysts installed. Five filters were used for each test. After each test the probe was brushed and rinsed with dichloromethane and the solution stored in a glass jar with a Teflon lined lid. Filters were stored in glass petri dishes. Impinger solutions were also stored in glass jars for possible future analysis.

Since sulphates were collected on the filters during catalyst tests, their removal was necessary for subsequent analysis. Following the dichloromethane extraction, filters were weighed and then extracted in iso-propyl alcohol to remove the sulphate fraction. Filters were then reconditioned and reweighed to determine the insoluble fraction on the filter. Sulphate fractions were also determined gravimetrically.

2.4 Dilution Tube Sampling System

A stainless steel dilution tube was originally designed as shown in Figure 3. In this system, compressed air is used to create a vacuum in the venturi section for exhaust sample withdrawal through the sample tube. A portion of the diluted exhaust could be sampled using the 125 mm sampling equipment. Fin strip heaters were installed to control diluent temperature if necessary. Previous results suggested that approximately 10 hours would be required to collect the 300 mg of

particulate material necessary for a complete 5 strain Ames assay. The excessive sampling time prompted a re-design of the dilution tube system.

Figure 4 illustrates the dilution tube used for tests given in this report. A Hi-Volume sampler blower was installed as the primary air mover. The venturi section was removed due to excessive restriction on the blower and a valve installed in the sample probe. Heat tape was wrapped around the sample probe to prevent, or minimize, sample losses. Diluent air was pre-filtered using a glass fiber filter. A 25 cm x 20 cm Pallflex filter was attached directly in front of the blower to collect particulates in the diluted exhaust. Filters were preconditioned and preweighed. With the 10 cm diameter tube the residence time of the diluted exhaust would be approximately 1 second (at 12.8:1 dilution ratio).

The carbon dioxide concentration was alternately measured in the raw and diluted exhaust gases. CO₂ results were used to determine the dilution ratio and subsequently the volume of exhaust gas sampled. The flowrate through the dilution tube was determined by measuring the blower static pressure with an inclined manometer. Calibration curves were obtained for the blower performance at various pressure drops.

Three dilution tube tests were conducted on uncontrolled diesel emissions at dilution ratios of 1 9:1, 8.6:1, and 15.4:1 for Test Nos. 1, 2 and 3 Dilute, respectively. Three additional tests were conducted for controlled emissions with Engelhard catalysts at dilution ratios of 12.9:1, 9.5:1, and 16.6:1 for Test Nos. 4, 5 and 6 Dilute, respectively. Results of these tests are given in the following section.

It was noted that the diluted exhaust temperature varied with the dilution ratio. Attempts were made to control the diluent temperature (using outside air at 14°C). However, consistent conditions could not be obtained. Results, therefore, must be evaluated with the diluted exhaust temperature being taken into consideration.

The same sulphate removal procedure as used in the 125 mm filter tests was necessary for the dilution tube filters. Sulphate extractions with iso-propyl alcohol were conducted on filters obtained from the controlled emissions test only.

2.5 Hot and Cold Filter Weighings

Concern regarding moisture adsorption on diesel particulate material prompted a need to determine the effects of conditioning sample filters. Eleven particulate test filters were weighed hot (directly from the 47 mm sampling system oven) and the weights recorded. The same eleven filters were conditioned in a room at 20°C and 50% relative humidity for 24 hours. Table 2 clearly indicates that the difference between the two weighing conditions is negligible.

Hot weighing conditions were conducted on three filters containing particulate material to determine if there was a change in the weight versus time during the weighing procedure. Results of these tests are given in Table 3 and show that once the filter is placed on the balance tray the weight does not change with time.

Air currents created by the balance door closing lead to a high initial filter weight. By the time the scale had stabilized, the filter may possibly have cooled down.

In addition, an "in-house" study of these Pallflex glass fibre filters Type T60A20 with a TFE binder have been evaluated for chemical resistance and hygroscopicity. They have been found to be inert to cyclohexane extraction and their weight consistent to within 0.1 mg whether weighed directly, conditioned for 24 hours, or heated to 200°C.

3. PARTICULATE EMISSION DATA

Engine and test conditions employed during the 125 mm and dilution tube testing are shown in Table 4. These are representative of the conditions after engine malfunction and repair. The following sub-sections

provide particulate emission data measured during tests of each of the three sampling systems employed.

3.1 Data Comparison Between 47 mm and 125 mm Sampling Systems

Table 5 shows the particulate concentrations measured using both the 47 mm and 125 mm sampling systems.

Referring to the 47 mm system results, there is a trend for increased organic insoluble matter to be collected as the sample gas temperature was increased, possibly due to reduced deposition on the sample line walls. These results were stabilized results obtained consistently during collection of each of the filters at any given temperature. Base line testing of this engine on a parallel CANMET contract provided the opportunity to compare ratios obtained using the normal sampling procedure (5) which is used for mass particulate emission determination, but is not suitable for the collection and determination of biological activity of particulates with that of the 47 mm and 125 mm systems described. At the load/speed condition employed during the present study, total particulate values of 48 mg/m^3 were observed using the conventional sampling methods. Total particulate determined with the 47 mm system was, however, consistently less than this value. Examination of the 125 mm system data shows why this occurs. A considerable portion of the particulate matter is deposited in the sampling probe, and when this is collected and added to the filter particulate, then the total particulate values obtained compare favourably with the expected value of 48 mg/m^3 .

The average soluble particulate loading for the 125 mm filter tests was 16.8 mg/m^3 , whereas the 47 mm filter tests showed a 5.35 mg/m^3 soluble particulate loading. Insoluble particulate loading averaged 28.4 mg/m^3 for the 125 mm filter tests and only Test No. 3 of the 47 mm filter tests compares favourably (27.1 mg/m^3).

Using the 125 mm filter tests as a reference, it appears that approximately 68% of the soluble particulate material is trapped in the 47 mm system

Teflon line. Based on these results, it was decided to perform mutagenic assays on the 125 mm filter system and dilution tube systems only.

3.2 125 mm Filter Sampling System Results

The comparison of the 125 mm filter system versus the 47 mm filter system reported in Section 3.1 was based on emission characteristics found before the engine malfunction. Results of the 125 mm filter tests after the engine malfunction are given in Table 6. Filters and probe rinses from these tests were analysed by the Ames test. The insoluble particulate loadings for Test Nos. 1 and 3 Raw are identical at 46.0 mg/m^3 . This shows that catalysts do not affect the carbon black (insoluble) material in diesel exhaust. The soluble particulate loading, however, is reduced by approximately 50% with the use of catalysts (i.e. 25.2 mg/m^3 versus 13.1 mg/m^3).

A significant amount (1727.3 mg) of sulphate was collected during the controlled emissions tests. Sulphates accounted for 85% of the total particulate material collected. The amount of sulphates on the filter can vary considerably according to the conditions of humidity and temperature during weighing. For this reason, all filters were weighed under controlled conditions of 50% relative humidity and 21°C temperature. However, because of the hygroscopic nature of H_2SO_4 , gravimetric determination of sulphates are not considered accurate. The objective here was simply to remove sulphates from the particulate matter in order to determine insoluble and SOF particulate.

The concentrations of soluble and insoluble material after the engine repair are considerably higher than those obtained before the engine malfunction. Results given in Table 7 may be more representative of emissions encountered in underground mines with similar engines.

3.3 Dilution Tube Sampling System Results

Results of the dilution tube tests for both controlled and uncontrolled diesel emissions are given in Table 8. The insoluble particulate concentration is highest for the lower dilution ratio of 8.6:1 for

uncontrolled and 9.5:1 for controlled emissions. It is suspected that the SOF for Test No. 6 Dilute is spurious. Insoluble particulate loadings with the dilution tunnel averaged 49.7 mg/m^3 which compare favourably with the 125 mm filter system average of 46.0 mg/m^3 . SOF concentrations averaged 14.5 mg/m^3 of exhaust for all 6 tests using the dilution tube. The SOF concentrations for uncontrolled emissions are somewhat less for the dilution tube tests than those found during the 125 mm filter tests.

Examination of the effect of the catalyst on particulate emissions, again shows that total particulate concentrations are reduced by the catalyst. However, an anomaly exists when the breakdown into insoluble and SOF is examined, since, contrary to expectations, the SOF is not reduced, the reduction occurring only in the insoluble fraction. There is no immediate explanation for this which could be a sampling or extraction artifact.

Sulphate concentrations accounted for approximately 87% of the total particulate material collected during the controlled emissions tests. Nitrogen dioxide concentrations found in the dilution tube were 3.0, 1.8 and 1.7 ppm, respectively, for dilution ratios of 9.5:1, 12.9:1 and 16.6:1. No NO_2 was found during the uncontrolled emissions tests.

4. SAMPLING SYSTEM COMPARISON

From the data given in this report it appears that the 47 mm sampling system has serious limitations. A significant amount of material (especially the SOF) is consistently retained in the Teflon sample line, during extended operation of the system (consecutive sampling of 52 filters), which should be included in the assessment of total particulate. These limitations prevented further analysis with the Ames test on the 47 mm filters.

The 125 mm filter system and dilution tube system results compare reasonably well for total particulate loadings. It generally appears that as the dilution ratio increases the percentage of soluble material

increases and the insoluble material decreases. A dilution ratio in excess of 10:1 is recommended, but it appears that there could be limitations to the dilution tube sampling system resulting from wall effects. This probably supports the EPA recommendation for a minimum tunnel diameter of 8 inches.

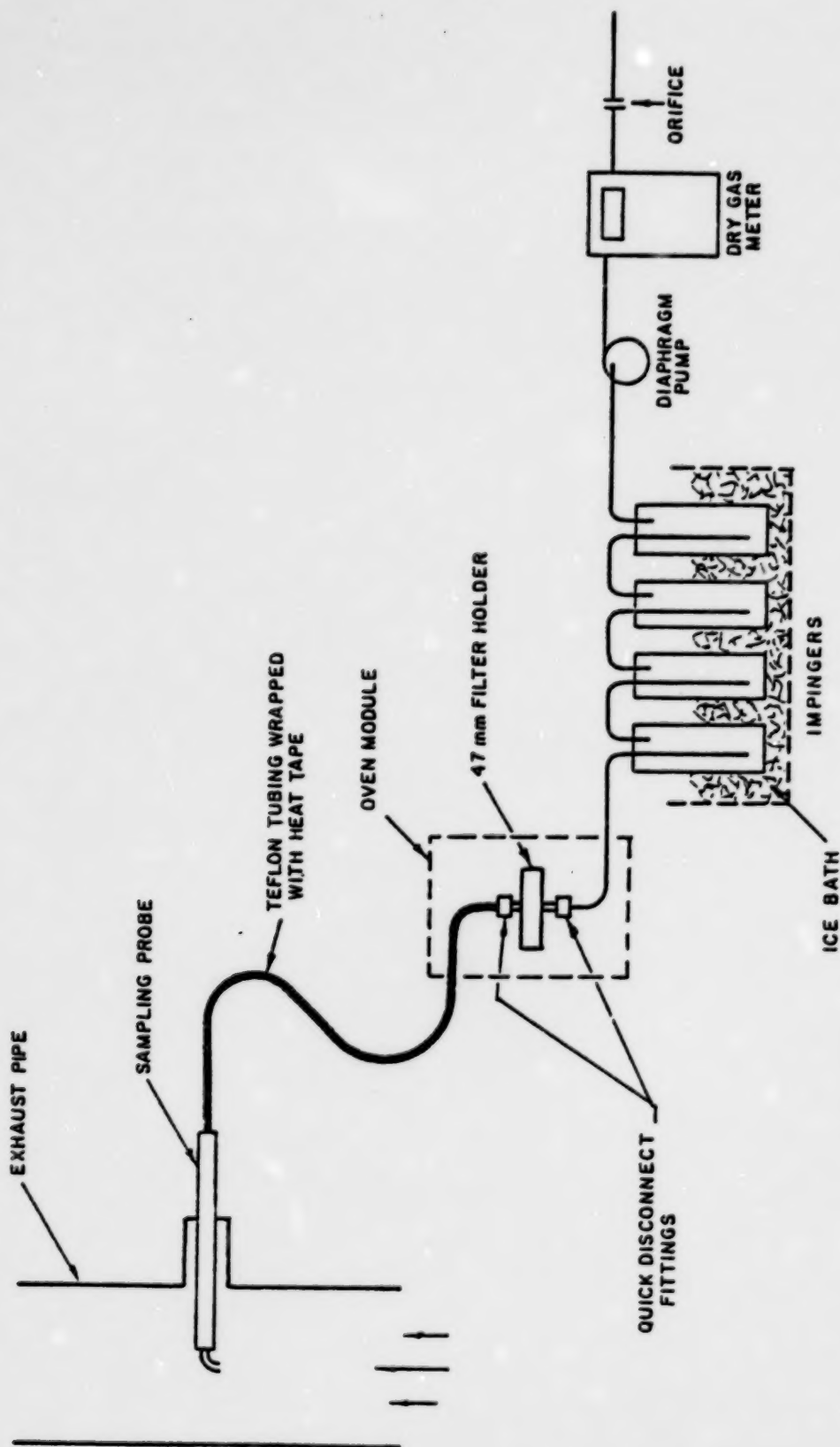


FIGURE I. 47mm PARTICULATE SAMPLING SYSTEM .

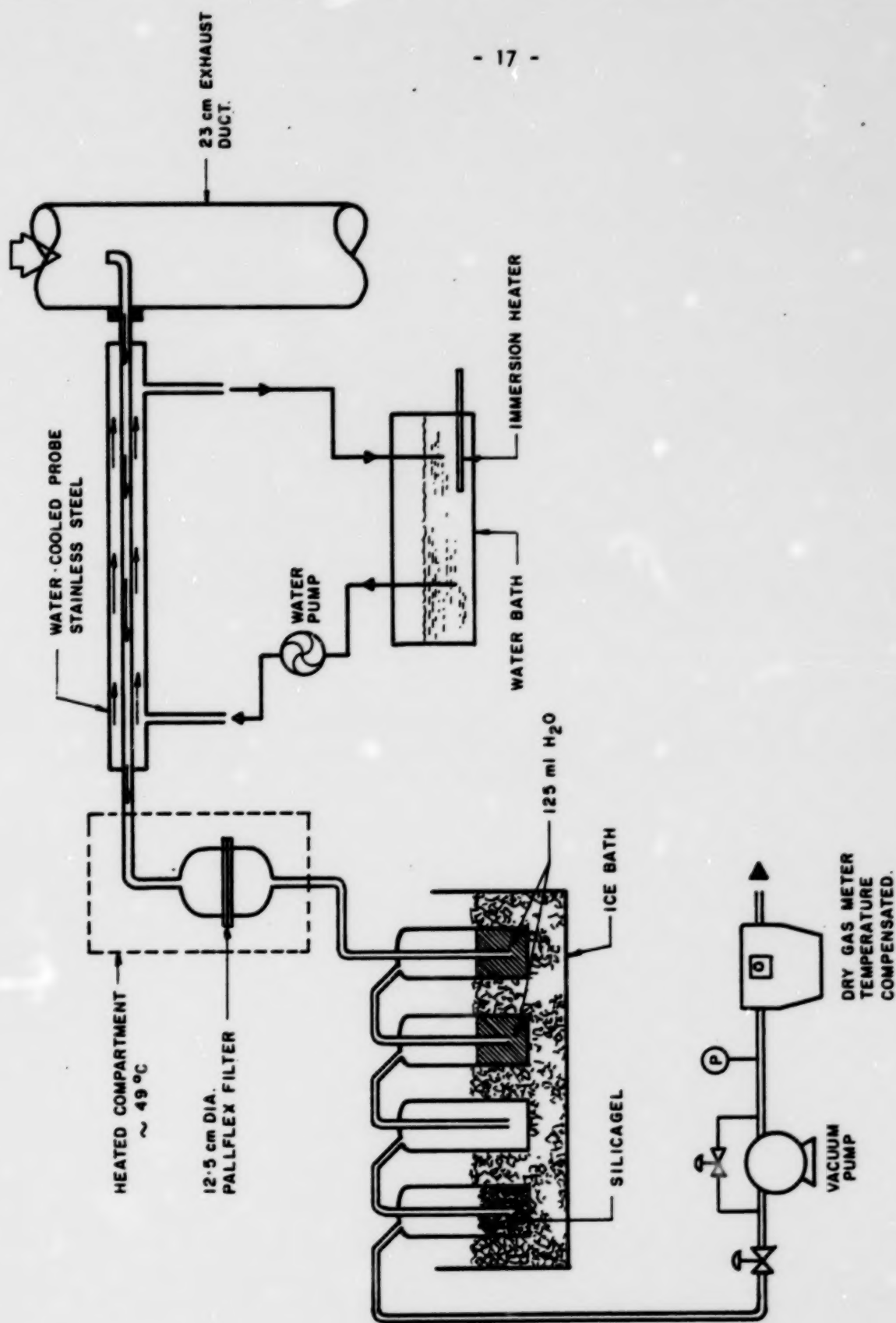


FIGURE 2: 12.5 cm SAMPLING SYSTEM

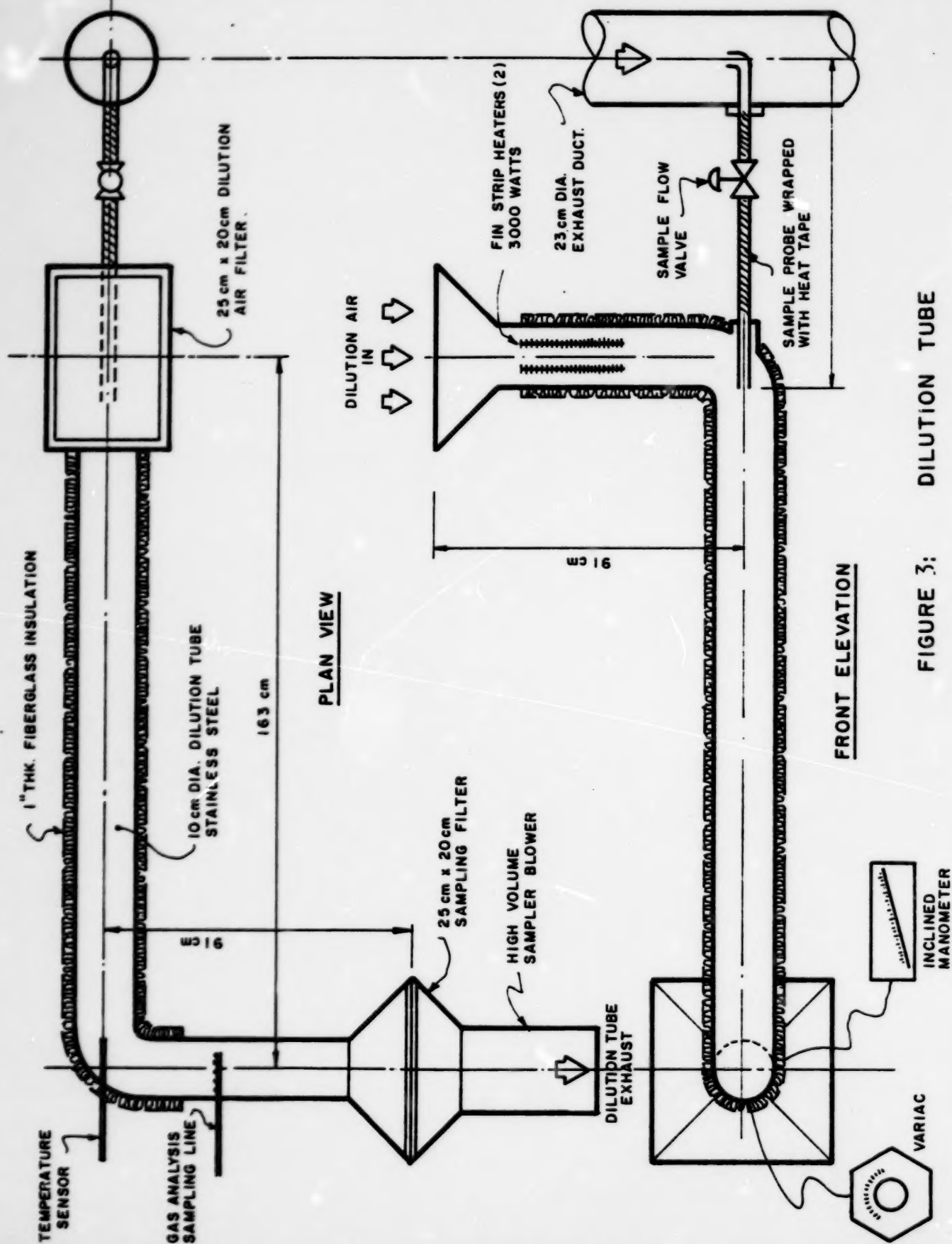


FIGURE 3: DILUTION TUBE

TABLE 1: Emissions from Monolithic Catalytic Purifiers
Engelhard Industries PTX60F

Speed rpm	Torque lb ft.	Load BHP	Catalyst Inlet Temp °F	Catalyst Outlet Temp °F	GASEOUS CONCENTRATIONS													
					% O ₂ Inlet and Outlet	% CO ₂ Inlet and Outlet	CO ppm			Total Hydrocarbons ppm			NO ppm		NO _x ppm		NO ₂ ppm	
							Inlet	Outlet	% Reduction	Inlet	Outlet	% Reduction	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
2200	298	125	1068	1085	6.5	10.2	176	50	72	60	12	80	610	570	620	590	10	20
2200	227	95	826	831	10.2	7.8	220	44	80	75	22	71	680	560	690	650	10	90
2200	149	62.5	620	627	13.4	5.7	172	40	77	65	18	72	660	470	680	630	20	160
1850	298	105	915	925	8.0	9.3	154	48	69	38	12	68	590	540	590	590	0	50
1850	227	80	719	730	10.6	7.5	124	38	69	46	16	65	650	430	670	560	20	130
1850	149	52.5	538	541	13.5	5.3	112	28	75	62	22	65	600	530	620	610	20	80
1500	298	85	844	835	9.1	8.6	132	36	73	16	3	81	540	450	540	520	0	70
1500	227	65	646	652	11.8	6.6	74	10	87	34	12	65	570	450	580	520	10	70
1500	149	42.5	463	463	14.5	4.7	52	5	90	30	10	67	510	500	540	530	30	30
1625	307	95	912	919	8.4	9.0	160	48	70	30	12	60	530	480	530	540	0	60

TABLE 2

Filter Weights - Hot Weighings vs Cold

2200 rpm 27° BTDC
86 Kw

Test	Initial Filter Weight g	Filter Weight with Particulate Hot		Filter Weight with Particulate Cold	
		g	mg	g	mg
1	.0726	.0912	18.6	.0912	18.6
2	.0683	.0864	18.1	.0864	18.1
3	.0736	.0926	19.0	.0927	19.1
4	.0718	.0893	17.5	.0894	17.6
5	.0714	.0883	16.9	.0884	17.0
6	-				
7	.0808	.1023	21.5	.1022	21.4
8	.0725	.0875	15.0	.0875	15.0
9	.0696	.0826	13.0	.0828	13.2
10	.0786	.0924	13.8	.0924	13.8
11	.0804	.0959	15.5	.0960	15.6
12	.0727	.0869	14.2	.0870	14.3

Filters were taken out of 55°C filter holders and weighed immediately (hot filters). The filters were then conditioned at 55% RH at 21°C then re-weighed 24 hours later.

TABLE 3

Time vs Filter Weight

Time (sec.)	Filter 1	Filter 2	Filter 3
0	.0913	.0926	.0893
5	.0911	.0928	.0894
10	.0913	.0925	.0893
15	.0912	.0924	.1892
20	.0911	.0925	.0892
25	.0912	.0926	.0893
30	.0911	.0926	.0893
35	.0910	.0925	.0892
40	.0910	.0925	.0892
45	.0912	.0927	.0893
50	.0912	.0926	.0893
55	.0911	.0926	.0892
60	.0912	.0926	.0893

TABLE 4: Engine and Test Conditions
Deutz F6L 714, 2200 RPM @ 86 KW

Date	Test No.	Emission Control Equipment	TEMPERATURES °C								Engine Back Pressure kPa	Barometric Pressure kPa	Humidity %	Fuel Consumption kg/h	O ₂ %	CO ₂ %	CO ppm	NOx ppm	NO ppm	NO ₂ ppm	Exhaust Flowrate m ³ /min
			Manifold		Stack	Exhaust Probe	Oven	Filter	Engine Air Intake												
			left	right																	
Feb. 22/81	1 Raw	None	480	478	445	52	55	52	27	0.5	100.7	51	9.8	7.9	220	620	620	0	7.97		
Feb. 23/81	2 Raw	None	478	475	448	53	54	52	22	0.5	99.2	58	9.6	8.1	230	620	620	0	7.84		
Mar. 19/81	3 Raw	PTX6DF	475	477	440	54	53	53	22	2.4	99.0	63	9.9	8.0	28	638	605	33	7.93		
Mar. 3/81	1 Diluted	None	480	479	438	290	NA	52	23	0.5	100.7	29	9.6	8.1	-	-	-	-	7.84		
Mar. 5/81	2 Diluted	None	478	475	445	340	NA	66	23	0.5	99.5	38	9.6	8.1	-	-	-	-	7.98		
Mar. 6/81	3 Diluted	None	475	478	439	318	NA	46	25	0.5	100.0	25	9.6	8.1	-	-	-	-	7.91		
Mar. 12/81	4 Diluted	PTX6DF	470	475	442	340	NA	53	25	2.4	99.3	29	9.8	8.1	34	635	612	23	7.88		
Mar. 16/81	5 Diluted	PTX6DF	480	478	443	335	NA	63	23	2.4	99.9	29	9.8	7.9	31	672	642	30	8.04		
Mar. 17/81	6 Diluted	PTX6DF	485	478	435	326	NA	40	20	2.4	98.5	25	10.0	7.8	31	661	633	28	8.06		

TABLE 5: 47 mm and 125 mm Test Comparison

Test No.	No. of Filters	Sampling System mm	Cumulative Sample Volume m	Sample Temp. °C	PARTICULATE CONCENTRATIONS mg/m ³							
					Filter			Probe			Filter + Probe	
					Soluble mg/m ³	Insoluble mg/m ³	Total mg/m ³	Soluble mg/m ³	Insoluble mg/m ³	Total mg/m ³	Soluble mg/m ³	Total mg/m ³
1	52	47	3.56	43	5.5	13.6	19.1	NA	NA	NA	NA	NA
2	35	47	2.35	141	4.9	19.0	23.9	NA	NA	NA	NA	NA
3	20	47	1.43	66	5.7	27.1	32.8	NA	NA	NA	NA	NA
4	6	125	5.56	50	9.0	17.4	26.4	7.6	10.6	18.2	16.5	44.5
5	8	125	10.52	50	9.1	17.6	26.7	8.1	11.1	19.2	17.1	45.9

Note: All data before engine malfunction.

TABLE 6: 125 mm Sampling Train Test Results

Date	Test	Emission Control Equipment	Volume Sampled m ³	Mass Collected			Concentration mg/m ³ exhaust			Emission Rate g/h		
				Soluble	Insoluble	Sulphate	Total	Soluble	Insoluble	Total	Soluble	Insoluble
Feb. 22/81	1 Raw	None	6.56	165.0	301.6	ND	466.6	25.2	46.0	71.2	12.0	22.0
Feb. 23/81	2 Raw	None	6.60	218.0	*	ND	323.8	33.0	*	*	15.5	*
Mar. 19/81	3 Raw	PTX6DF	6.39	83.6	294.2	1727.3	2031.6	13.1	46.0	59.1	6.22	21.9
												28.1

* Data not available

NOTE: Sulphates excluded from concentration and emission rate calculations

ND - None Detected

TABLE 7: Particulate Emission Rates for Deutz F6L714

Test No.	Speed RPM	Load kW	PARTICULATE EMISSION RATES								
			Insoluble			Soluble			Total		
			g/kW-h	g/h	g/kg fuel	g/kW-h	g/h	g/kg fuel	g/kW-h	g/h	g/kg fuel
1	2200	99	0.228	22.6	0.818	0.082	8.16	0.296	0.310	30.7	1.11
2	2200	99	0.222	22.1	0.799	0.059	5.88	0.213	0.282	27.9	1.01
		avg.	0.225	22.4	0.808	0.070	7.02	0.255	0.296	29.3	1.06
3	2200	75	0.164	12.4	0.608	0.120	9.03	0.445	0.284	21.4	1.05
4	2200	75	0.175	13.2	0.651	0.097	7.32	0.360	0.273	20.5	1.01
5	2200	75	0.184	13.8	0.682	0.095	7.18	0.354	0.279	21.0	1.04
		avg.	0.174	13.1	0.647	0.104	7.84	0.386	0.278	20.9	1.03
6	2200	50	0.268	13.4	0.848	0.225	11.2	0.711	0.493	24.6	1.56
7	2200	50	0.266	13.3	0.842	0.198	9.88	0.625	0.464	23.2	1.46
8	2200	50	0.134*	6.73*	0.426*	0.172	8.60	0.544	0.306*	15.3	0.970*
		avg.	0.267	13.4	0.845	0.198	9.89	0.626	0.478	23.9	1.51
9	1900	93	0.268	25.0	1.04	0.070	6.49	0.272	0.337	31.4	1.32
10	1900	93	0.245	22.8	0.961	0.061	5.70	0.240	0.306	28.6	1.20
11	1900	93	0.278	25.9	1.08	0.064	6.02	0.253	0.342	31.9	1.34
		avg.	0.264	24.6	1.02	0.065	6.07	0.255	0.328	30.6	1.28
12	1900	70	0.185	13.0	0.742	0.086	6.08	0.348	0.272	19.0	1.09
13	1900	70	0.174	12.2	0.698	0.082	5.80	0.332	0.257	18.0	1.03
14	1900	70	0.214	15.0	0.858	0.064	4.48	0.256	0.278	19.5	1.11
		avg.	0.191	13.4	0.766	0.077	5.45	0.312	0.269	18.8	1.08
15	1900	47	0.238	11.2	0.816	0.230	10.8	0.787	0.467	22.0	1.60
16	1900	47	0.242	11.4	0.830	0.172	8.12	0.592	0.415	19.5	1.42
17	1900	47	0.274	12.8	0.941	0.132	6.22	0.454	0.407	19.1	1.40
		avg.	0.251	11.8	0.862	0.178	8.38	0.611	0.429	20.2	1.47
18	1600	78	0.392	30.7	1.62	0.064	5.02	0.266	0.456	35.7	1.89
19	1600	78	0.401	31.4	1.66	0.075	5.89	0.312	0.476	37.3	1.97
20	1600	78	0.424	33.2	1.76	0.147	11.5	0.610	0.570	44.7	2.36
		avg.	0.406	31.8	1.68	0.095	7.47	0.396	0.500	39.2	2.07

* Values not included in data averaging
Suspected spurious result

TABLE 8: Dilution Tube Results

Date	Test	Emission Control Equipment	Dilution Ratio	Dilution Tube Gas Temperature °C	Volume Sampled m ³	Total Particulate mg	Soluble mg	Insoluble mg	Sulphate mg	ENGINE EMISSION RATES AND CONCENTRATIONS						NO ₂ in Dilution Tube ppm
										Soluble Concentrations mg/m ³	Insoluble Concentrations mg/m ³	Total Concentrations mg/m ³	Emission Rate g/hr			
													Soluble	Insoluble	Total	
Mar. 3/81	1 Dilute	none	12.9:1	52	73.49	413.6	107.4	306.2	-	18.9	53.8	72.7	8.89	25.31	34.1	<1
Mar. 5/81	2 Dilute	none	8.6:1	66	38.02	310.1	44.6	265.5	-	10.1	60.0	70.1	4.84	28.73	33.6	<1
Mar. 6/81	3 Dilute	none	15.4:1	46	80.06	350.5	93.0	257.5	-	17.9	49.5	67.4	8.50	23.49	32.0	<1
Mar. 12/81	4 Dilute	PT6DF	12.9:1	53	56.85	1983.0	80.0	199.6	1703.4	18.2	45.3	63.5	8.61	21.42	30.0	1.8
Mar. 16/81	5 Dilute	PT6DF	9.5:1	63	44.48	2213.2	60.5	222.4	1930.3	12.9	47.5	60.4	6.22	22.91	29.2	3.0
Mar. 17/81	6 Dilute	PT6DF	16.6:1	40	80.48	2234.3	47.6	203.4	1988.3	8.8	41.9	50.7	4.26	20.26	24.5	1.7

* Total emission rates do not include sulphates

** Stack concentration and emission rates are calculated from dilution tube loadings

5. AMES MUTAGENICITY ASSAYS

5.1 Proposed Work

As part of the overall objectives of the programme the following work was proposed.

1. Select and evaluate an extraction method for obtaining the soluble organic fraction of diesel particulates, for use with Ames mutagenicity assay.
2. Determine the storage properties of the particulate extract.
3. Determine the Ames mutagenic activity of the particulate emissions from a Deutz F6L 714 diesel engine.
4. Determine the Ames mutagenic activity of the particulate emissions from a Deutz F6L 714 diesel engine equipped with an Engelhard PTX catalytic exhaust purifier.
5. Select the most sensitive tester strain and assay conditions and suggest a simplified method for conducting future cost efficient sample programmes.

5.2 Experimental Procedures

5.2.1 Extraction Method - Materials and Supplies

- Collection filters 47 mm 125 mm and 8" x 10" Pallflex Teflon-coated glassfibre film T60A20.
- Extraction solvent Dichloromethane (DCM) Caledon Laboratories Ltd. This solvent meets ACS reagent grade specifications. In addition, it has been dried and distilled in glass to remove all residue.
- Extraction thimbles, cellulose, single thickness, Whatman, pre-extracted with DCM.
- Pyrex 250 ml soxhlet extraction apparatus.
- Electrothermal 6 place heating unit.

- Vacuum rotary evaporator.
- Pyrex boiling beads.
- Bioassay solvent, Dimethylsulphoxide (DMSO) Spectrophotometric grade, Schwarz/Mann.

5.2.2 Method

Particulate samples collected on Pallflex filters were placed in an extraction thimble to prevent the insoluble particulates from becoming part of the sample. The thimbles containing the sample were then placed into a 250 ml soxhlet extraction apparatus with 160 ml of DCM solvent and extraction was carried out for 16 hours at a rate of 3 to 4 exchanges per hour.

After 16 hours, the extraction thimble containing the insoluble material was removed and the solvent was returned to the boiling flask. The DCM solvent was then concentrated to approximately 100 ml by distillation.

Determination of Concentration of Extractables

At this point 5 ml of the concentrated extract (100 ml) was transferred to a weighed aluminum dish. The DCM solvent was removed under nitrogen and the aluminum dish was reweighed. The concentration was determined by:

$$\begin{aligned} \text{wt of 5 ml Dry} \div 5 &= \text{wt per ml Extractable} \\ \times \text{the number of ml of solvent} &= \text{wt of extract} \end{aligned}$$

Solvent Exchange

The ~100 ml of DCM solvent was then further concentrated by distillation to approximately 20 ml and allowed to cool to room temperature. 25 ml of DMSO was added. The volume of DMSO added varied depending on the concentration of extract required and the amount of sample required to carry out the Ames assays. The sample was then placed on a rotary evaporator at 25°C under vacuum and the remaining 20 ml of the DCM Extraction solvent was removed.

5.3 Ames Bioassay - Background Information

The assay uses five mutant strains (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) of the bacterium Salmonella typhimurium. Each strain contains a point mutation in the gene coding for histidine biosynthesis. This results in a defective enzyme in the histidine biosynthetic pathway. Therefore, the mutant Salmonella strains (his^-) are unable to grow on media which do not contain histidine (e.g. minimal media). In addition to the histidine mutation, each tester strain contains structural modifications. One such modification enables agents to permeate more easily into the bacteria. A second alteration interferes with the bacteria's genetic repair mechanism. This permits the action of the mutagen (reversion of his^- to his^+) to be expressed and thus detected by the assay.

Exposure of the tester strains to substances which can alter genetic activity (mutagens) results in some of the mutant cells (his^-) reverting back to their wild type (his^+) state. In the process, the bacteria regain the ability to synthesize histidine and now will show up as colonies on histidine-free media.

Mammalian-microsomal enzymes, the source of which is predominantly rat liver, are often used in the assay's reaction mixture. These enzymes serve as part of a metabolic activation complex which also includes compounds that aid in electron transport and energy production. The activation complex is needed as many substances which are non-mutagenic outside animal systems are structurally altered to a mutagenic form by metabolic processes within animal tissues. Since Salmonella do not inherently possess these metabolic enzymes the reaction mixture must be supplemented. However, microsomal enzymes have inhibitory as well as activating properties. Therefore, when a substance is tested it must be tested both with and without the metabolic activation complex. If not, the activation complex might inhibit the response of a mutagen which does not normally require activation to express its mutagenic effect.

The frequency at which bacteria revert to the wild type (his^+) depends upon several factors, one of which is the mutagenic potency of the agent tested. Mutagenic agents which are highly potent will induce a greater number of bacteria to revert to his^+ than will agents which produce less mutagenic damage. Therefore, the number of colonies induced by a mutagenic substance, at a given dose, provides a measure of the potential hazard of the substance.

5.3.1 Ames Bioassay Method

The plate incorporation assay was conducted as described by Ames et al using Salmonella tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. All tester strains were obtained directly from Dr. B.N. Ames (University of California, Berkeley). Stock cultures were maintained and characterized according to procedures described by Ames et al.⁶

Briefly, 2 ml of molten top agar containing trace amounts of histidine and biotin were added to sterile tubes and the tubes were placed into a dry block maintained at 45°C. To each tube was then added 0.1 ml of a Salmonella tester strain, a given concentration of the sample extract between 0.1 and 0.5 ml per tube, and 0.5 ml of S-9 mix. In one half of the cases the S-9 mix was deleted. Each tube was mixed and poured onto minimal agar plates. Uniform distribution of the top agar on the surface of the plates was accomplished by gently tilting and rotating the plates. Plates were then incubated in the dark at 37°C. After 48 hours the colonies (histidine revertants) in both the treated and control plates were scored. In addition, the density of the background lawn was visually evaluated for toxicity.

Quintuplicate platings were conducted for all spontaneous revertant negative controls. Triplicate platings were conducted for each concentration of particulate extract tested.

The metabolic activation mixture contained the following substances combined in the quantities (per ml) indicated, with the final concentration of the mixture set at 0.5 ml per plate:

- I Aroclor 1254-induced rat liver homogenate (Catalogue Number 8360-03, Litton Bionetics) 0.04 ml;
- II $MgCl_2$ 8 μ moles, KCl 33 μ moles;
- III Glucose-6-phosphate, monosodium salt (Sigma Catalogue Number G7879) 5 μ moles;
- IV NADP Sodium Salt (Sigma Catalogue Number N0505) 4 μ moles;
- V Sodium phosphate Buffer, pH 7.4 100 μ moles.

The final mixture is referred to in this report as the metabolic activation mixture or S-9 mix.

5.3.2 Controls

All quality assurance procedures as described by Ames et al and the Environmental Mutagens Society Committee⁸ on the Ames test were followed. Negative controls for each of the five tester strains were conducted in quintuplicate, both with and without metabolic activation.

Positive controls were conducted for each of the five tester strains with and without metabolic activation to verify that the assay was functioning properly. A single concentration of a positive control compound was tested and the results compared to the historical value to ensure the assay was functioning normally.

In addition, all experiments were conducted in a laminar flow biological exhaust hood under yellow lights. When sufficient sample was available, each extract was tested in triplicate at a minimum of five doses.

5.3.3 Criteria for Positive and Negative Results

There is a finite number of histidine revertants characteristic for each tester strain and these spontaneous background revertants are routinely measured on the negative control plates. A substance is suspected to be a mutagen when the number of revertants produced on the treated plates exceeds the number of background revertants by a

critical amount. A quantitation of the mutagenic response is made using the mutagenic activity ratio (MUTAR) described by Commoner⁷ and calculated by the formula:

$$\text{MUTAR} = E - C/C_{AV}$$

where E is the number of revertants produced in response to the test compound; C is the number of spontaneous revertants for the respective tester strain; E - C is the revertants in excess of the spontaneous (negative) control level and C_{AV} is the average number of spontaneous revertants calculated from the historical record of negative control plates.

The probability of correctly classifying a compound as mutagenic or non-mutagenic based on MUTAR values using the criteria set by Commoner is shown in Table 9.

For this report a mutagenic activity ratio of 2.5 was selected as it provides the highest probability for correctly identifying a non-mutagen while still maintaining a high probability of detecting a mutagen. This value was the value recommended by Commoner.

The demonstration of a dose-related increase in histidine independent colonies is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increase may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

If a chemical produces a response in a single test that cannot be reproduced in one or more additional tests, the initial positive data lose their significance.

5.4 Results and Discussion

5.4.1 Extraction Procedure

Three solvents - benzene, cyclohexane and dichloromethane - were evaluated and found to be extremely toxic to the Salmonella tester strains.

The problem of obtaining sufficient sample using the 47 mm collection system to allow evaluation of the different solvent systems forced the selection of one method, in order to obtain at least some mutagenicity data.

The system selected was that used by EPA and others for the evaluation of Ames mutagenicity data for above-ground diesel application.

A blank run using the DCM solvent, Pallflex collection filter, extraction thimbles and all components such as wash bottles, nylon brushes, etc., at amounts 4 or 5 times higher than it was expected would be used for actual testing, were put through a simulated extraction procedure, concentrated and solvent exchanged with DMSO and submitted to the Ames assay. The results of the blank runs showed no signs of mutagenic activity or increase in toxicity to the Salmonella tester strains.

It was later discovered that traces of DCM solvent were being carried over during the solvent exchange procedure and may have been responsible for some high levels of toxicity observed for some of the samples in subsequent work.

Table 10 presents Ames mutagenicity data for the 125 mm sampling system prior to the engine breakdown.

Table 10 shows a considerable portion of the Ames activity was present in the probe rinse portion of the sample and in order to obtain the total Ames activity, it is necessary to combine the probe rinse and particulate filters.

Table 11 presents the activity obtained for tester strains TA 98 and TA 100 without metabolic activation for a period of up to 20 days of storage of the sample after extraction and solvent exchange with DMSO.

The sample in DMSO was stored in the frozen state at +4°C in the dark and the results for strain TA 98 without activation show an increase in toxicity with time. The results for tester strain TA 100 without metabolic activation show an increase in mutagenicity with time. Because of the amount of sample available and because of the problems with the engine, these results were not thought to be conclusive. Further planned testing was prevented by the engine breakdown and the attempt to evaluate the storage properties of samples further was abandoned because of time constraints.

All future samples were run the morning following sample collection to ensure a minimum of storage related changes.

Table 12 presents the historical negative control data for each of the five tester strains both with and without metabolic activation used to calculate the mutagenic activity ratio, MUTAR.

Table 13 presents the average of the positive control data conducted with each Ames assay and confirms that the assay system was functioning properly during all testing.

Table 14 presents data for an experiment conducted to select a range of concentrations to be tested. Because of the sample volume limitation and the requirement to test on all five tester strains with and without metabolic activation, it was not possible to conduct and evaluate the effect of toxicity using the three methods normally employed when conducting Ames assays of unknown samples.

Toxicity is defined as the killing of the Salmonella tester strain(s) by the sample material being tested. Toxicity is important because it can affect the interpretation of results. It is necessary that the concentration range selected covers two or three log concentrations and that the highest dose selected shows slight toxicity.

Toxicity was determined visually, using a binocular microscope, of the background lawn on sample test plates. The sample concentrations were plated with 10^8 cells on minimal agar plates which contained trace

amounts of histidine and biotin. The trace amount of histidine allowed mutant cells to divide once or twice in order to express their mutation. However, once the trace histidine has been depleted, only those cells which have become histidine independent will form colonies. The growth on the plate of the remaining non-colony forming cells, i.e. those which have not reverted back to a histidine independent state, represent the background lawn. By visual evaluation of the density of the background lawn, the dose at which the test material begins to show toxicity can be estimated. This estimation was made by comparing the lawn on those plates containing no sample extract (negative control plates) with the background lawn on those plates containing various concentrations of the sample extract. All concentrations of sample extract that showed signs of toxicity to the tester strains using this method are indicated in the Tables as TOX. At least one concentration of toxicity should be examined because of the relationship of mutagenicity and toxicity to ensure a positive mutagenic response has not been missed. Only those concentrations where no toxicity was evident were used for assessing the mutagenic activity of the samples. In some cases, the toxicity appeared at low concentrations and may have been due to incomplete removal of DCM solvent. This suspicion should be confirmed before finally standardizing the extraction method.

Table 14 indicates that samples should be tested over a range of 0 to 2000 μg per plate whenever sufficient sample is available.

The results obtained for the Ames mutagenic activity of the Deutz F6L 714 engine at the four different sample dilutions used are presented in the upper parts of Tables 15, 16, 17 and 18. The effect of the PTX catalytic exhaust purifier on the mutagenic activity is presented in the lower part of Tables 13, 16, 17 and 18. Tables 15, 16, 17 and 18 are summary tables of the Ames mutagenicity assays, the raw data are presented in Appendix I, Tables 21 to 28.

Tables 15, 16, 17 and 18 show that all sample dilutions tested both with and without PTX catalyst are:

1. Negative and non-mutagenic to tester strain TA 1535 in the presence and absence of metabolic activation.
2. Tester strains TA 1537, TA 1538 and TA 98 show a positive mutagenic response both in the presence and absence of metabolic activation.
3. Tester strain TA 100 shows a negative mutagenic response without PTX at 15.4 to 1 dilution ratio and a positive mutagenic response with PTX at a 16.6 to 1 dilution both with and without metabolic activation. All other dilution tunnel experiments showed negative results both with and without PTX catalyst. The 125 mm sampling system showed slightly positive results both with and without PTX catalyst.

The mutagenic activity of the Deutz diesel emissions observed for the five tester strains is consistent with that previously reported for other types of heavy duty diesel engines, i.e. they are negative for strain TA 1535 with and without activation, but they are positive for strains TA 1537, TA 1538 and TA 98 with and without metabolic activation. In most cases, there is a decrease in mutagenic activity in the presence of metabolic activation and there are signs of less toxicity when the metabolic activation is present. These three strains all respond to frameshift type mutagens and would detect polyaromatic hydrocarbons (PAHs) and nitrated PAHs.

The results obtained with tester strain TA 100 are both positive and negative with a high sensitivity to toxicity. This strain responds to both frameshift and base pair substitution mutations. These results are not unexpected, as the literature indicates that the best results for TA 100 are normally obtained after sample fractionation. This strain is normally positive after fractionation because of the removal of the toxic components which interfere with the results for the unfractionated sample.

Our results indicate that tester strain TA 98, while not always the most sensitive for the detection of the mutagenic activity of the soluble extract from diesel particulates, is probably as good as any of the 3 positive strains, and since most data in the literature collected were on strain TA 98 it has been selected to evaluate the Deutz engine with and without PTX catalyst.

Table 19 presents a summary of the mutagenic activity ratios and the maximum revertants per microgram of soluble extract for the various sampling systems with and without catalyst. At the maximum dilution tested the sample with catalyst appears more mutagenic. At the 12.9 to 1 dilution ratio the sample without catalyst appears more mutagenic. At a low dilution ratio 8.6 to 9.5 to 1 there is little difference in the mutagenic activity either with or without catalyst. The 125 mm sampling system shows the engine with catalyst to be more mutagenic.

Table 20 presents a summary of the particulates and soluble organics per cubic metre of exhaust emissions and gives the maximum revertants per cubic metre for tester strain TA 98 with and without metabolic activation. These results increase proceeding from the most dilute sample to the least dilute, the latter being the 125 mm sampling system. Table 20 shows similar results to Table 17 with respect to the mutagenic activity shown with and without catalyst treatment of the exhaust.

5.5 Conclusions

1. The DCM extraction method is suitable for preparation of samples of diesel particulate emissions for Ames bioassay.
2. Care should be taken during the solvent exchange procedure to remove all of the DCM so as not to cause increases in toxicity to the Salmonella tester strains.
3. All samples tested in the Ames assay showed a negative response to tester strain TA 1535.

4. All samples tested in the Ames assay showed positive mutagenic responses to tester strains TA 1537, TA 1538 and TA 98 both with and without metabolic activation.
5. Tester strain TA 100 showed a positive/negative response not unexpected for the unfractionated samples.
6. While it appears that the results obtained with PTX exhaust treatment are slightly more mutagenic than those obtained without PTX treatment, there is insufficient data and replication to determine quantitatively how much more mutagenic the "with PTX" treatment sample may be. It is certain, however, that they are not very much more mutagenic than those samples obtained without PTX catalyst treatment of the exhaust emissions.
7. It is probably sufficient to conduct further Ames testing of this engine and catalyst combination using the TA 98 tester strain only, with and without metabolic activation.

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TABLE 9

Probability of Correctly Classifying a Mutagen as a Mutagen and a Non-Mutagen as a Non-Mutagen at Various Mutagenic Activity Ratios. All Values were taken from Commoner.⁷

MUTAR	0.7	1.5	2.5
	<u>Probability</u>		
<u>Case I</u>			
Probability of correctly classifying a mutagen	0.95	0.82	0.81
<u>Case II</u>			
Probability of correctly classifying a non-mutagen	0.20	0.82	0.95

Calculated from optimal tester strain, optimal concentration of test substance and metabolic (S-9) activation when necessary.

TABLE 10

Evaluation of 125 mm Sample System

	µg/Plate Extract	TA 1535 - S-9	TA 1537 - S-9	TA 1538 - S-9	TA 98 - S-9 + S-9		TA 100 - S-9 + S-9	
DCM Solubles Probe Rinse Conc. of DMSO soln 1560 µg/ml	780	TOX	TOX	TOX	200	150	280	215
	390	26	TOX	132	170	150	180	200
	156	26	TOX	66	100	80	160	180
	78	26	TOX	33	100	70	160	160
	39	26	TOX	24	60	50	160	160
	15.6	26	TOX	12	30	42	160	160
	7.8 3.9	26 26	7 8	12 12	30 30	36 35	160 160	160 160
		26 Neg.	8 TOX	12 +	30 +	35 +	160 -	160 -
DCM Solubles 16 hr Extraction of Filter Particulates Conc. of DMSO soln 1900 µg/ml	950	TOX	TOX	TOX	400	280	TOX	440
	475	26	81	TOX	320	200	440	330
	190	26	36	106	200	120	280	240
	95	26	27	118	170	100	260	220
	47.5	26	14	32	60	50	190	150
	19	26	9	28	48	51	200	150
	9.5 4.7	26 26	8 8	21 13	36 30	31 30	204 198	150 150
		26 Neg.	8 +	12 +	30 +	35 +	170 -	150 -
DCM Solubles Probe Rinse plus 16 hr Extraction of Filter Particulates Conc. of DMSO soln 3000 µg/ml	1500	This part of the experiment was omitted in favour of obtaining storage data Time 0 days			680	480	820	TOX
	750				780	520	550	310
	300				600	280	330	230
	150				510	220	280	200
	75				213	180	200	194
	30				161	89	188	180
	15 7.5				102 34	61 55	180 180	180 180
					34 +	37 +	180 +	170 -

TABLE 11

	Conc. ug/Plate Extract	TA 98 - 5-9	TA 100 - 5-9
0 Days Storage at 4°C	1500	680	820
	750	780	550
	300	600	330
	150	510	280
8 Days Storage at 4°C	1500	430	860
	750	590	1130
	300	470	820
	150	450	710
14 Days Storage at 4°C	1500	TOX	
	750	660	
	300	460	
	150	360	
20 Days Storage at 4°C	1500	TOX	
	750	760	
	300	560	
	150	480	

After Sample Extraction with DCM for 16 hours
and solvent exchange with DMSO, samples were
stored frozen at 4°C.

TABLE 12: Historical negative control data for each of the five Salmonella tester strains with and without metabolic activation.

Tester strain	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Metabolic Activation (S-9)	-	+	-	+	-	+	-	+	-	+
Spontaneous Revertant Negative Control Average C _{AV}	23	13	6	9	22	22	32	36	179	159

TABLE 13: Historical positive control data for each of the five Salmonella tester strains with and without metabolic activation.

Tester Strain	S-9	Compound	Concentration µg/Plate	Histidine Revertants
TA 1535	-	Sodium Azide	1 µg	550
TA 1537	-	2 Nitrofluorene	25 µg	180
TA 1538	-	2 Nitrofluorene	2 µg	478
TA 98	-	2 Nitrofluorene	2 µg	358
TA 100	-	2 Nitrofluorene	2 µg	596
TA 1535	+	2 Aminoanthracene	5 µg	183
TA 1537	+	2 Aminoanthracene	5 µg	138
TA 1538	+	2 Aminoanthracene	1 µg	396
TA 98	+	2 Aminoanthracene	1 µg	698
TA 100	+	2 Aminoanthracene	1 µg	1208

TABLE 14: An experiment to determine the approximate maximum concentration range that should be screened for mutagenicity

Concentration µg/Plate Extract	TA 1538 without S-9 mix	(Average)	TA 98 without S-9 mix	(Average)	TA 100 without S-9 mix	(Average)
10,900	T0X		T0X		T0X	
5,450	T0X		T0X		T0X	
2,180	T0X		T0X		T0X	
1,090	530 620 640	(597)	846 800 853	(833)	1218 1263 1206	(1229)
540	451 423 386	(420)	522 489 490	(500)	673 661 617	(650)
218	223 233 228	(228)	393 428 379	(406)	532 562 537	(544)
109	165 155 135	(152)	196 214 237	(216)	419 426 426	(424)
54	120 119 125	(121)	127 135 126	(129)	361 233 341	(312)
22	29 40 50	(40)	103 101 65	(90)	184 156 179	(173)
10	27 30 32	(30)	60 55 47	(54)	183 202 177	(187)
0	22 20 18 21 20 (20)		28 25 26 28 23 (26)		177 151 159 192 157 (167)	

TABLE 15: Ames mutagenic activity of the exhaust emission from a Deutz diesel F6L 714 collected using the dilution sampling system. The upper part of the Table presents data obtained without exhaust treatment. The lower part of the Table presents data obtained when the engine was equipped with an Engelhard PTX catalytic exhaust purifier.

Highest Dilution Ratio

Salmonella tester strains		µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Metabolic Activation (S-9 mix)			-	+	-	+	-	+	-	+	-	+
Sample 15.4 to 1 dilution ratio without PTX exhaust treatment		1420	TOX	17	TOX	60	TOX	201	TOX	178	TOX	177
		700	25	16	52	65	156	129	150	132	214	161
		280	22	18	29	25	81	74	111	97*	162	159
		140	15	11	18	20	73	44	79	56	160	160
		71	23	17	12	19	30	22	39	38	160	157
		28	23	12	7	14	16	18	32	35	157	154
Spontaneous Revertant Background Controls		14	29	13	8	11	17	18	32*	35	163	150
		0	22	14	7	9	21	20	26	33	159	149
Maximum Mutagenic Activity Ratio			0.1	0.3	7.5	6.2	6.1	8.2	3.9	4.0	0.3	0.2

Salmonella tester strains		µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Metabolic Activation (S-9 mix)			-	+	-	+	-	+	-	+	-	+
Sample 16.6 to 1 dilution ratio with PTX exhaust treatment	644	16	18	150	93	TOX	TOX	2666	1500*	911	874	
	322	20	11	70	39	1422	532	887	569	1051	914	
	128	22	16	51	19	703	231	517	260	838	625	
	64	24	18	33	15	616	121	456*	174	660	483	
	32	22	20	16	8	148	59	139	111	356	277	
	12	21	14	8	5	79	38	89	68	287	241	
	6	25	8	10	9	64	26	55	52	267	207	
Spontaneous Revertant Background Controls		0	20	13	8	9	25	23	36	44	196	180
Maximum Mutagenic Activity Ratio		0.2	0.5	23.6	9.3	63.5	23.1	82.2	40.4	4.8	4.6	

TABLE 16: Ames mutagenic activity of the exhaust emissions from a Deutz diesel F6L 714 collected using the dilution sampling system. The upper part of the Table presents data obtained without exhaust treatment. The lower part of the Table presents data obtained when the engine was equipped with an Engelhard PTX catalytic exhaust purifier.
Second Highest Dilution Ratio

Salmonella tester strains	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 12.9 to 1 dilution ratio without PTX exhaust treatment	1625	TOX	14	TOX	TOX	TOX	400	590	340	420	290
	812	20	14	130	110	350	300	470	300	400	300
	325	25	13	90	60	200	200	260	200	270	210
	162	25	12	40	20	140	130	160	130	210	160
	81	20	14	20	11	100	90	120	70	190	160
	40	20	14	10	8	60	50	100	60	200	140
	16	25	14	5	7	40	50	60*	50	180	160
	8	20	13	5	7	40	30	40	50*	190	160
Spontaneous Revertant Background Control	0	25	14	6	8	27	18	28	35	190	160
Maximum Mutagenic Activity Ratio		0	0	20.6	11.3	14.7	17.4	17.6	8.5	1.3	0.9

Salmonella tester strains	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 12.9 to 1 dilution ratio without PTX exhaust treatment	1210	TOX	14	TOX	TOX	TOX	TOX	TOX	TOX	TOX	494
	605	26	14	94	45	TOX	TOX	216	173	TOX	464
	242	23	15	54	37	TOX	TOX	176	123	291	329
	121	21	14	33	33	57	86	98	80	309	315
	60	32	12	10	15	36	62	75	70	190	183
	24	25	13	7	8	16	27	50	49	167	192
	12	26	13	8	8	13	23	55*	52*	197	208
Spontaneous Revertant Background Control	0	26	13	7	8	21	20	40	34	188	146
Maximum Mutagenic Activity Ratio		0.3	0.3	14.5	4.1	3.8	3.0	5.5	3.9	0.7	2.2

TABLE 17: Ames mutagenic activity of the exhaust emissions from a Deutz diesel F6L 714 collected using the dilution sampling system. The upper part of the Table presents data obtained without exhaust treatment. The lower part of the Table presents data obtained when the engine was equipped with an Engelhard PTX catalytic exhaust purifier.

Lowest Dilution Ratio

Salmonella tester strains		TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Metabolic Activation (S-9 mix)	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 8.6 to 1 dilution ratio without PTX exhaust treatment	676	TOX	TOX	TOX	TOX	TOX	TOX	385	297	TOX	214
	338	22	13	130	69	249	227	261	240	320	210
	135	28	12	45	33	106	64	208	131	247	172
	67	21	14	28	21	52	44	155	79	228	157
	34	23	12	13	13	26	53	79	49	160	140
Spontaneous Revertant Background Controls	13	29	13	5	10	23	30	62*	48	177	150
	7	23	12	6	11	19	27	32	49*	166	140
Maximum Mutagenic Activity Ratio		0.3	0.2	20.8	6.7	10.4	13.6	11.1	7.3	0.9	0.5

Salmonella tester strains		TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Metabolic Activation (S-9 mix)	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 9.5 to 1 dilution ratio without PTX exhaust treatment	916	TOX	TOX	TOX	TOX	TOX	TOX	531	373	398	260
	458	25	15	235	135	162	233	541	388	298	212
	183	26	17	99	71	128	153	341	220	232	180
	91	24	14	82	34	101	116	281*	113	207	192
	46	23	13	41	15	59	59	93	51	169	158
Spontaneous Revertant Background Controls	18	22	14	21	15	50	34	65	64*	165	177
	9	24	14	13	10	39	32	54	39	160	165
Maximum Mutagenic Activity Ratio		0.1	0.1	38.0	14.0	6.7	9.0	15.8	11.2	1.3	0.6

TABLE 18: Ames mutagenic activity of the exhaust emissions from a Deutz diesel F6L 714 collected using the dilution sampling system. The upper part of the Table presents data obtained without exhaust treatment. The lower part of the Table presents data obtained when the engine was equipped with an Engelhard PTX catalytic exhaust purifier.

Salmonella tester strains	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 125 mm System filter + probe without PTX exhaust treatment	2750	TOX	13	TOX	183	TOX	470	665	375	TOX	TOX
	1375	18	15	116	104	TOX	401	521	422	810	658
	550	24	14	44	67	206	261	424	338	732	421
	275	20	15	37	36	143	137	347	200	500	377
	137	20	14	25	32	127	126	243	111	365	240
	55	26	14	22	17	92	73	174*	91	323	208
Spontaneous Revertant Background Control	27	25	15	11	12	57	39	93	67	245	202
	14	20	16	10	11	42	39	66	57*	245	198
Maximum Mutagenic Activity Ratio	0	25	13	5	7	25	16	36	31	202	180
		0	0.2	18.5	19.6	8.2	20.6	19.7	10.9	3.4	3.0

Salmonella tester strains	µg/ Plate Extract	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		-	+	-	+	-	+	-	+	-	+
Sample 125 mm System, filter + probe without PTX exhaust treatment	1200	TOX	21	TOX	603	1559	1200	1760	1833	TOX	967
	600	TOX	19	696	262	1075	980	1069	886	1078	737
	240	21	14	257	107	594	272	946	407	847	424
	120	23	15	105	50	268	151	457	211	599	290
	60	20	12	54	25	103	60	229	110	202	182
	24	16	11	19	16	51	36	117	72	181	183
Spontaneous Revertant Background Control	12	23	12	9	13	47	29	87	49	154	166
	0	25	11	6	10	22	26	31	41	190	160
Maximum Mutagenic Activity Ratio		0	0.8	115.0	65.9	69.9	53.4	54.0	49.8	5.0	5.0

TABLE 19: Ames Mutagenic Activity with and without PTX exhaust treatment for tester strain TA 98 in the presence and absence of metabolic activation.

Sample Dilution	Catalyst	Mutar TA 98		Max. Rev/ μ g TA 98	
		-	+	-	+
15.4	No	3.9	4.0	0.43	0.23
16.6	Yes	8.22	40.4	6.56	2.26
12.9	No	17.6	8.5	2.0	1.9
12.9	Yes	5.5	3.9	1.25	1.5
8.6	No	11.1	7.3	2.54	2.0
9.5	Yes	15.8	11.2	2.70	1.83
0	No	19.7	10.9	2.51	1.90
0	Yes	54.0	49.8	7.2	4.0

TABLE 20: Results for with and without PTX using TA 98 data

Sample	Catalyst	Total Particulates mg/M ³	Soluble Particulates mg/M ³	Tester strain TA-98			
				Maximum Revertants per µg		Maximum Revertants per M ³	
				- S-9	+ S-9	- S-9	+ S-9
15.4	No	67.8	18.5	0.43	0.23	8 x 10 ³	4.3 x 10 ³
16.6	Yes	51.5	8.3	6.56	2.26	54.4 x 10 ³	18.8 x 10 ³
12.9	No	72.2	19.4	2.0	1.90	38.8 x 10 ³	36.9 x 10 ³
12.9	Yes	63.2	18.1	1.25	1.50	22.6 x 10 ³	27.2 x 10 ³
8.6	No	70.5	10.3	2.54	2.0	26.2 x 10 ³	20.6 x 10 ³
9.5	Yes	60.8	13.3	2.70	1.83	35.9 x 10 ³	24.3 x 10 ³
0	No	71.1	25.1	2.51	1.9	63.1 x 10 ³	47.8 x 10 ³
0	Yes	317.9	13.1	7.20	4.0	94.2 x 10 ³	52.3 x 10 ³

APPENDIX I

AMES MUTAGENICITY RAW DATA TABLES

for samples tested after the engine was repaired

TABLE 21: Ames activity of the untreated exhaust collected using the 125 mm sampling system

Conc. µg/plate Extract	Activa- tion	TA 1535 Histidine Revertants (Average)	TA 1537 Histidine Revertants (Average)	TA 1538 Histidine Revertants (Average)	TA 98 Histidine Revertants (Average)	TA 100 Histidine Revertants (Average)
2750	-	TOX	TOX	TOX	577 606 812	TOX
1375	-	18 11 26 (18)	132 115 101 (116)	TOX	465 453 646 (521)	822 800 809 (810)
550	-	21 28 22 (24)	44 42 46 (44)	204 193 222 (206)	362 379 532 (424)	670 854 672 (732)
275	-	19 21 20 (20)	34 28 49 (37)	142 104 182 (143)	361 292 387 (347)	502 435 563 (500)
137	-	22 18 20 (20)	19 32 23 (25)	135 113 132 (127)	254 241 234 (243)	351 397 347 (365)
55	-	28 18 31 (26)	31 14 20 (22)	90 90 94 (92)	180 155 187 (174)	328 315 325 (323)
27	-	26 19 30 (25)	11 12 11 (11)	67 45 60 (57)	103 96 79 (93)	247 253 235 (245)
14	-	16 21 24 (20)	8 13 9 (10)	44 40 41 (42)	63 71 63 (66)	261 247 228 (245)
0	-	25 23 25 (25)	4 9 4 (5)	31 22 17 (25)	26 33 30 (36)	207 211 244 (202)
		22 30	6 3	30 25	50 39	185 165
2750	+	12 13 15 (13)	174 214 161 (183)	449 455 507 (470)	403 346 376 (375)	TOX
1375	+	12 19 15 (15)	103 100 108 (104)	360 401 443 (401)	406 456 404 (422)	646 703 625 (658)
550	+	13 14 16 (14)	41 84 76 (67)	256 279 247 (261)	351 310 352 (338)	398 442 422 (421)
275	+	18 12 15 (15)	36 36 35 (36)	121 146 144 (137)	177 216 207 (200)	372 411 347 (377)
137	+	15 15 13 (14)	30 34 32 (32)	111 134 133 (126)	93 95 145 (111)	251 239 230 (240)
55	+	13 13 16 (14)	16 15 20 (17)	60 85 75 (73)	88 97 89 (91)	216 209 198 (208)
27	+	18 12 14 (15)	9 9 18 (12)	42 47 27 (39)	66 69 65 (67)	188 253 165 (202)
14	+	20 20 8 (16)	9 10 13 (11)	38 33 45 (39)	58 51 61 (57)	221 202 170 (198)
0	+	21 8 13 (13)	11 3 7 (7)	14 18 16 (16)	34 25 36 (31)	197 201 179 (180)
		13 11	6 8	16 15	24 38	164 159

- without 5-9; + with 5-9; 0 concentration is the spontaneous background of control for the tester strain;
TOX = sample concentration toxic to tester strain.

TABLE 22: Ames activity of the untreated exhaust collected at a 12.9 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants
1625	-	TOX	TOX	TOX	590 579 602	485 341 434
812	-	23 13 23	150 118 122	340 346 365	460 461 489	404 392 404
325	-	18 30 27	74 76 120	107 230 183	258 268 254	242 239 329
162	-	24 24 26	39 40 41	137 131 152	165 159 156	202 192 236
81	-	25 20 17	23 21 17	106 91 103	135 112 112	190 176 204
40	-	20 20 20	9 10 11	58 69 53	94 114 92	190 190 221
16	-	24 25 25	5 6 5	50 44 25	58 60 63	196 166 176
8	-	19 20 21	5 5 5	39 39 40	48 37 35	205 179 186
0	-	21 24 25	8 7 5	17 20 32	24 31 35	162 199 184
		24 31	6 4	29 37	20 30	198 207
1625	+	12 14 14	TOX	404 400 396	398 339 286	346 327 202
812	+	12 12 18	89 121 120	290 315 295	303 292 305	316 269 316
325	+	12 12 14	54 63 62	189 217 194	204 191 205	190 220 222
162	+	12 12 12	20 20 20	125 134 131	135 120 135	176 156 148
81	+	12 14 15	12 13 9	98 85 87	75 68 67	174 140 166
40	+	12 14 15	8 7 8	55 47 48	47 84 51	137 127 156
16	+	14 14 14	7 7 7	52 42 55	55 39 55	159 154 167
8	+	12 13 14	6 7 8	30 30 30	52 57 40	160 160 159
0	+	12 15 15	10 6 9	29 17 14	33 30 36	171 131 161
		14 13	9 7	15 15	35 41	185 152

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;

TOX = sample concentration toxic to tester strain.

TABLE 23: Ames Activity of the untreated exhaust collected at an 8.6 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Histidine Revertants (Average)	Histidine Revertants (Average)	Histidine Revertants (Average)	Histidine Revertants (Average)	Histidine Revertants (Average)
6.76	-	TOX	TOX	TOX	354 428 373 (385)	TOX
338	-	18 25 24 (22)	155 108 126 (130)	265 248 233 (249)	226 333 224 (261)	314 295 351 (320)
135	-	25 35 25 (28)	58 23 53 (45)	103 109 106 (106)	202 228 195 (208)	221 268 252 (247)
6.7	-	20 27 16 (21)	31 26 26 (28)	44 66 45 (52)	168 142 154 (155)	253 213 219 (228)
134	-	22 23 23 (23)	13 16 9 (13)	28 26 25 (26)	69 73 94 (79)	160 167 152 (160)
13	-	27 31 28 (29)	5 6 4 (5)	26 23 20 (23)	66 71 50 (62)	176 170 185 (177)
7	-	21 21 26 (23)	4 6 7 (6)	23 12 23 (19)	27 42 28 (32)	191 166 143 (166)
0	-	18 24 29 (21)	4 4 4 (5)	23 21 29 (20)	29 41 27 (29)	136 163 161 (157)
		18 18	4 4 8	15 12	28 21	154 171
6.76	+	TOX	TOX	269 405 289 (321)	304 279 308 (297)	208 183 252 (214)
338	+	10 14 14 (13)	71 72 65 (69)	240 231 210 (227)	244 201 275 (240)	202 198 232 (210)
135	+	11 10 15 (12)	31 40 27 (33)	39 87 65 (64)	122 132 138 (131)	170 151 194 (172)
6.7	+	13 12 16 (14)	14 26 24 (21)	32 55 45 (44)	84 79 74 (79)	163 151 158 (157)
34	+	13 12 10 (12)	11 10 17 (13)	56 46 56 (53)	58 58 31 (49)	145 147 130 (140)
13	+	11 18 10 (13)	6 12 13 (10)	25 39 26 (30)	39 54 53 (48)	151 147 152 (150)
7	+	14 9 13 (12)	11 10 13 (11)	25 24 32 (27)	58 47 42 (49)	157 128 135 (140)
0	+	8 12 15 (12)	10 5 10 (9)	24 25 24 (22)	38 34 29 (35)	112 176 146 (142)
		12 13	9 9	21 16	32 40	137 138

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;
TOX = sample concentration toxic to tester strain.

TABLE 24: Ames activity of the untreated exhaust collected at a 15.4 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535 Histidine Revertants (Average)	TA 1537 Histidine Revertants (Average)	TA 1538 Histidine Revertants (Average)	TA 98 Histidine Revertants (Average)	TA 100 Histidine Revertants (Average)
1420	-	TOX 27 21 27 (25)	TOX 68 48 41 (52)	TOX 193 159 115 (156)	TOX 141 148 161 (150)	TOX 181 261 201 (214)
700	-	22 13 31 (22)	25 31 31 (29)	72 70 102 (81)	130 107 95 (111)	148 178 161 (162)
280	-	17 16 13 (15)	25 19 10 (18)	90 66 62 (73)	73 82 82 (79)	168 145 167 (160)
140	-	23 21 26 (23)	16 11 10 (12)	25 29 36 (30)	36 33 49 (39)	161 161 157 (160)
71	-	27 15 28 (23)	5 11 5 (7)	18 23 7 (16)	37 27 33 (32)	160 160 151 (157)
28	-	25 34 28 (29)	6 8 8 (8)	14 18 19 (17)	31 39 26 (32)	175 163 151 (163)
14	-	21 23 23 (22)	6 7 7 (7)	21 21 15 (21)	25 26 31 (26)	158 165 148 (159)
0	-	24 21	5 10	19 27	18 30	179 143
1420	+	17 18 17 (17)	70 61 48 (60)	255 239 109 (201)	159 187 187 (178)	165 195 172 (177)
700	+	19 13 16 (16)	60 59 76 (65)	88 176 123 (129)	92 140 163 (132)	128 182 180 (161)
280	+	19 19 15 (18)	21 27 28 (25)	83 73 67 (74)	102 101 88 (97)	159 146 172 (159)
140	+	10 12 10 (11)	23 18 19 (20)	41 39 53 (44)	40 57 71 (56)	160 161 159 (160)
71	+	16 15 19 (17)	17 19 20 (19)	18 28 20 (22)	36 41 38 (38)	160 175 136 (157)
28	+	10 8 18 (12)	15 14 11 (14)	25 15 13 (18)	27 38 39 (35)	141 157 164 (154)
14	+	13 13 13 (13)	12 10 11 (11)	16 19 18 (18)	35 37 33 (35)	128 149 173 (150)
0	+	16 16 16 (14)	8 9 11 (9)	19 18 21 (20)	35 38 31 (33)	145 91 183 (149)
		17 5	11 8	21 21	27 34	179 147

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;

TOX = sample concentration toxic to tester strain.

TABLE 25: Ames activity of the exhaust after treatment with the PTX catalytic exhaust purifier sample collection using the 125 mm sampling system

Conc. µg/plate Extract	Activa- tion	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants
		(Average)	(Average)	(Average)	(Average)	(Average)
1200	-	TOX	TOX	1242 1598 1836	1750 2084 1447	1062 1098 1075
600	-	TOX	636 726 726	1147 1069 1010	1426 637 1145	840 852 850
240	-	18 21 23	250 241 281	555 582 645	730 1145 962	585 630 581
120	-	21 22 25	130 108 78	226 291 288	437 503 430	224 178 204
60	-	20 21 18	42 55 66	107 105 98	250 226 211	203 183 157
24	-	19 16 14	20 18 18	47 59 46	98 130 123	163 153 147
12	-	17 20 32	10 8 0	34 58 48	81 85 95	198 201 167
0	-	31 33 23	7 7 5	22 19 21	39 24 27	200 187
		17 21	4 7	18 28	31 32	
1200	+	26 17 19	357 776 675	1200 1100 1300	2100 2100 1300	952 986 962
600	+	23 19 16	191 196 398	871 976 1100	928 861 850	654 764 793
240	+	16 15 10	128 72 122	274 203 338	397 312 510	469 362 440
120	+	14 18 13	58 45 47	126 188 140	234 236 170	297 305 268
60	+	7 14 15	14 31 30	53 65 61	119 105 105	158 209 178
24	+	10 11 13	20 17 11	29 45 35	77 63 76	165 217 167
12	+	13 11 13	16 17 7	28 29 29	48 52 48	158 168 173
0	+	10 12 10	7 11 14	21 21 31	43 44 42	151 160 157
		13 10	5 11	22 35	37 40	172 161

- without 5-9; + with 5-9; 0 concentration is the spontaneous background of control for the tester strain;

TOX = sample concentration toxic to tester strain.

TABLE 26: Ames activity of the exhaust after treatment with an Engelhard catalytic exhaust purifier sample collection at a 12.9 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants
1210	-	TOX	TOX	TOX	TOX	TOX
605	-	14 17 12 (14)	99 89 95 (94)	76 130 107 (104)	269 193 168 (216)	306 300 266 (291)
242	-	30 19 28 (26)	60 61 42 (54)	66 55 51 (57)	202 156 169 (176)	281 342 303 (309)
121	-	17 20 31 (23)	43 25 32 (33)	42 42 25 (36)	133 76 85 (98)	198 211 162 (190)
60	-	14 24 25 (21)	10 15 4 (10)	18 13 17 (16)	84 72 68 (75)	166 185 151 (167)
24	-	37 35 24 (32)	4 10 7 (7)	14 10 15 (13)	54 44 53 (50)	192 190 209 (197)
12	-	23 23 30 (25)	3 10 11 (8)	20 20 21 (21)	58 61 46 (55)	210 174 211 (188)
0	-	19 26 22 (26)	6 6 8 (7)	21 21 23	38 41 43 (40)	186 162
1210	+	TOX	TOX	TOX	TOX	467 575 439 (494)
605	+	16 10 25 (17)	46 44 45 (45)	126 74 59 (86)	175 200 145 (173)	415 476 500 (464)
242	+	14 14 14 (14)	31 37 44 (37)	84 56 46 (62)	116 150 102 (123)	312 312 364 (329)
121	+	16 14 15 (15)	20 53 25 (33)	29 22 31 (27)	75 72 92 (80)	326 295 323 (315)
60	+	14 14 15 (14)	14 18 14 (15)	24 20 24 (23)	83 61 67 (70)	168 181 199 (183)
24	+	12 16 7 (12)	10 8 7 (8)	15 25 21 (20)	47 53 48 (49)	212 187 178 (192)
12	+	11 13 16 (13)	8 10 7 (8)	20 21 19	61 52 45 (52)	212 226 188 (208)
0	+	15 16 13 (13)	8 8 6 (8)	20 21 19	31 22 37 (34)	150 170 149 (146)

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;

TOX = sample concentration toxic to tester strain.

TABLE 27: Ames activity of the exhaust after treatment with an Engelhard catalytic exhaust purifier sample collection at a 9.5 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants	Histidine Revertants
		(Average)	(Average)	(Average)	(Average)	(Average)
916	-	TOX	TOX	TOX	508 533 552	373 433 361
458	-	29 27 18	294 252 160	177 145 166	514 546 563	317 297 253
183	-	31 24 23	88 105 104	100 129 156	290 366 368	254 214 229
91	-	30 23 19	94 75 77	95 81 127	256 291 296	176 242 203
46	-	21 23 25	41 41 41	66 69 41	96 91 93	165 170 174
18	-	25 27 13	26 25 15	45 49 57	58 75 62	171 181 144
9	-	24 22 26	13 16 10	45 34 36	62 47 52	170 166 144
0	-	24 19 28	2 7 5	17 17 22	31 41 30	190 146 169
		21 25	11 10	27 21	37 36	166 160
916	+	20 16 15	TOX	204 156 233	449 350 321	243 267 276
458	+	16 13 15	118 118 168	262 255 184	348 456 359	186 236 216
183	+	12 17 21	71 67 74	122 195 142	239 187 235	186 164 191
91	+	17 12 13	34 36 32	127 108 112	152 82 100	205 184 186
46	+	13 9 17	14 15 16	52 63 62	57 46 56	141 164 169
18	+	14 14 13	12 14 18	24 33 44	70 60 63	174 184 175
9	+	9 16 16	7 15 8	34 36 25	33 46 39	180 156 158
0	+	15 16 19	8 11 14	22 17 17	27 21 33	197 131 146
		17 13	11 5	21 28	34 39	143 169

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;
TOX = sample concentration toxic to tester strain.

TABLE 28: Ames activity of the exhaust after treatment with an Engelhard catalytic exhaust purifier sample collection at a 16.6 to 1 dilution ratio

Conc. µg/plate Extract	Activa- tion	TA 1535 Histidine Revertants (Average)	TA 1537 Histidine Revertants (Average)	TA 1538 Histidine Revertants (Average)	TA 98 Histidine Revertants (Average)	TA 100 Histidine Revertants (Average)
644	-	13 10 24 (16)	169 130 150 (150)	TOX 104 1436 1426 (1422)	2441 2900 2657 (2666)	1072 615 1046 (911)
322	-	19 20 20 (20)	95 91 25 (70)	588 929 594 (703)	886 918 859 (887)	1036 1049 1069 (1051)
128	-	19 23 24 (22)	46 50 56 (51)	626 591 632 (616)	552 362 637 (517)	835 861 819 (838)
64	-	26 24 22 (24)	29 40 31 (33)	130 150 164 (148)	435 523 410 (456)	606 667 708 (660)
32	-	31 14 20 (22)	17 15 17 (16)	76 85 75 (79)	128 147 141 (139)	326 378 366 (356)
12	-	21 21 20 (21)	8 9 7 (8)	74 48 70 (64)	110 92 65 (89)	297 293 272 (287)
6	-	23 32 19 (25)	8 10 12 (10)	18 31 22 (25)	60 40 64 (55)	260 256 285 (267)
0	-	19 21 20 (20)	4 11 7 (8)	26 28	40 27 37 (36)	172 156 215 (196)
		20 20	7 9		37 39	260 175
644	+	18 19 17 (18)	90 79 110 (93)	TOX 442 670 485 (532)	1300 1500 1700 (1500)	842 740 1041 (874)
322	+	9 10 13 (11)	40 53 24 (39)	240 247 208 (231)	494 520 694 (569)	857 935 951 (914)
128	+	20 20 8 (16)	23 17 17 (19)	129 124 110 (121)	207 271 302 (260)	699 537 640 (625)
64	+	18 18 20 (18)	17 12 15 (15)	42 70 65 (59)	168 153 201 (174)	513 443 493 (483)
32	+	20 20 21 (20)	8 7 8 (8)	40 47 28 (38)	120 97 117 (111)	303 298 230 (277)
12	+	13 15 15 (14)	11 1 4 (5)	29 24 25 (26)	64 76 64 (68)	241 265 217 (241)
6	+	7 8 9 (8)	6 11 10 (9)	18 22 20 (23)	58 48 50 (52)	222 223 175 (207)
0	+	16 18 13 (13)	13 8 6 (9)	32 21	52 44 33 (44)	179 156 173 (180)
		8 8	9 7		52 41	213 177

- without S-9; + with S-9; 0 concentration is the spontaneous background of control for the tester strain;

TOX = sample concentration toxic to tester strain.

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